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Bovine digital dermatitis

A spirochetal skin disease of polytreponemal aetiology

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National Veterinary Institute

Technical University of Denmark

DTU

Bovine digital dermatitis: A spirochetal skin disease of polytreponemal aetiology

Tim K. Jensen

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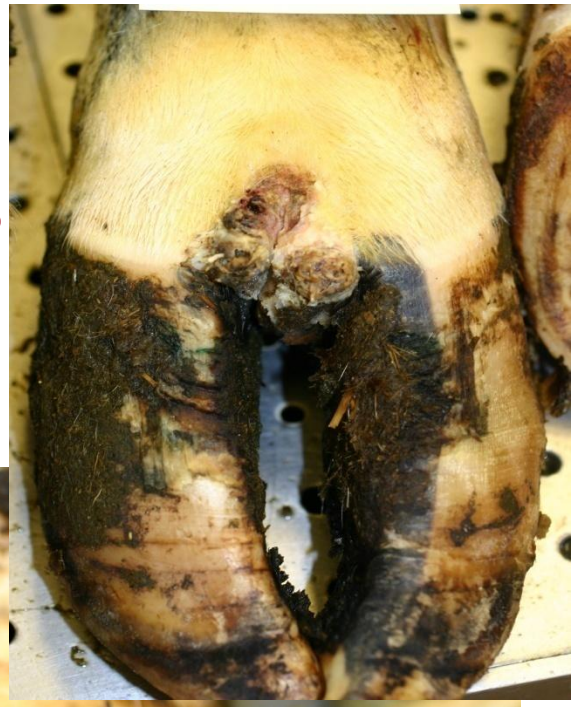
Oslo, June 4th 2010

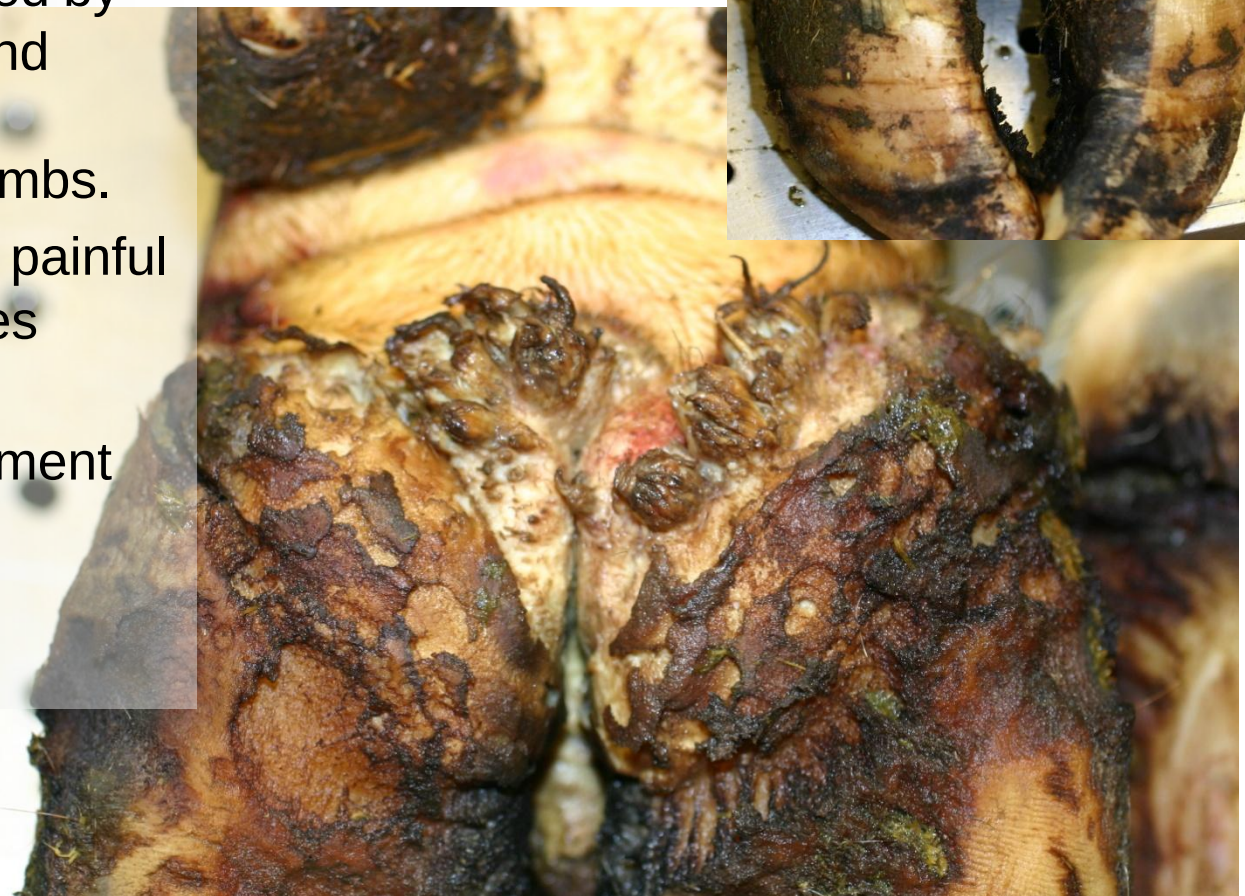
Bovine digital dermatitis

- 1991: DVM
- 1991 – 1995: PhD-student in Veterinary pathology. Spirochetal colitis in pigs
- 1995 – present: Veterinary Institute, Copenhagen.
- 2005: Project idea.
- 2007-2009: Digital dermatitis in Danish dairy cattle, **Danish Cattle Federation**

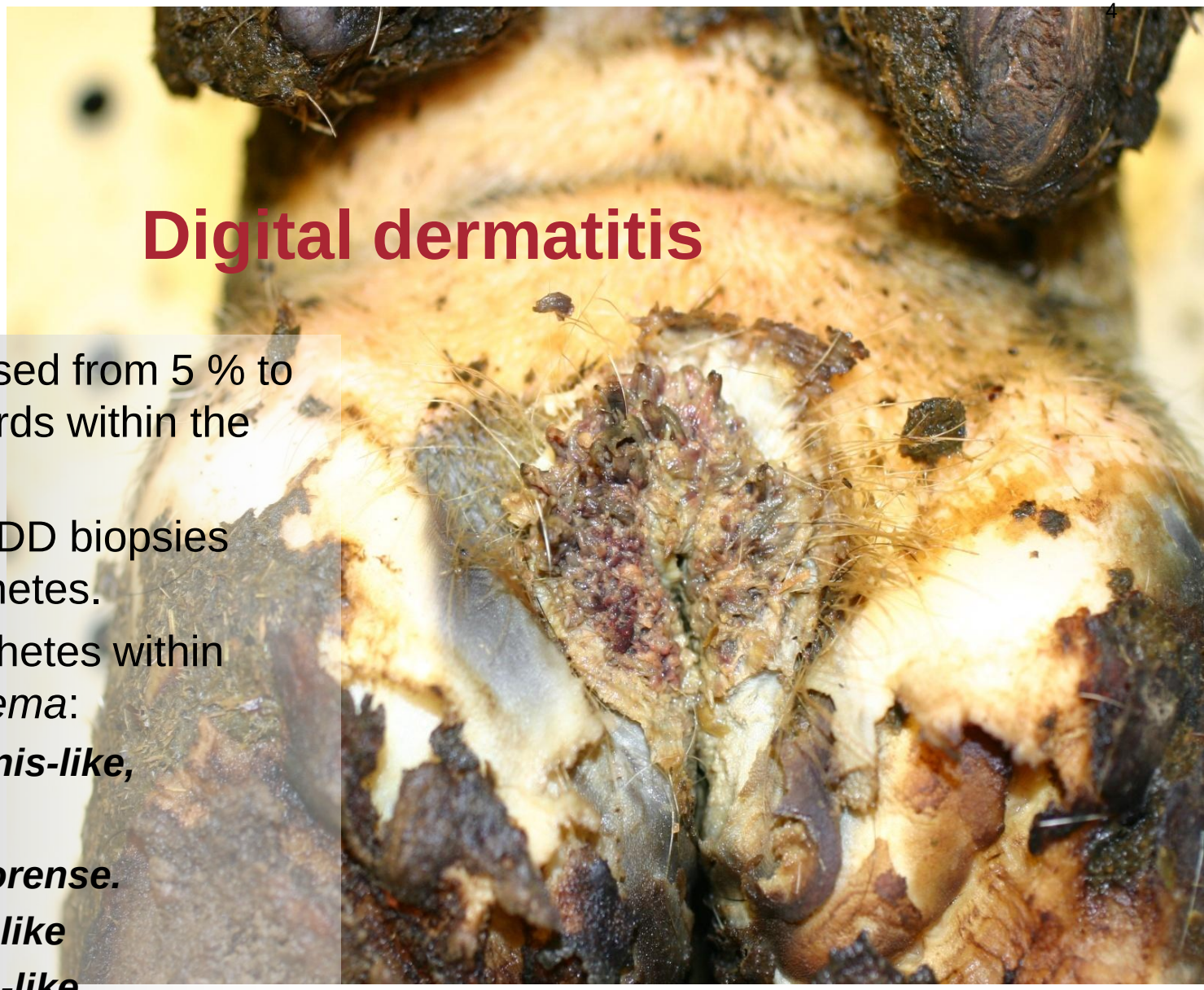
Bovine digital dermatitis



- Bovine digital dermatitis (DD), an inflammatory skin disease of cattle, is characterized by focal, circumscribed, and papillomatous lesions localized to the lower limbs.
- DD is acute to chronic, painful at palpation, and causes lameness and wasting.
- DD is sensitive to treatment with antibiotics.
- No virus identified.



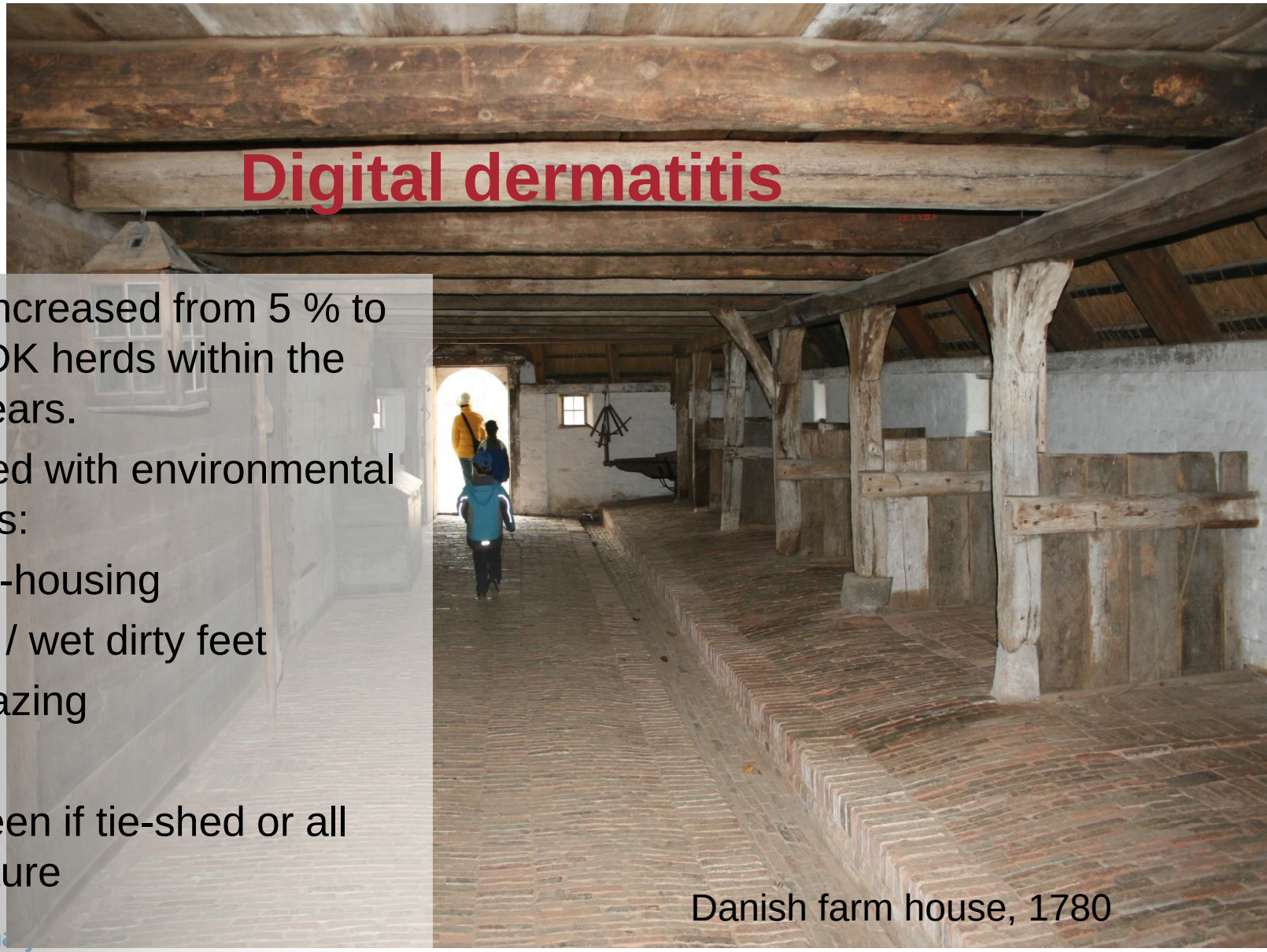
Digital dermatitis



- DD has increased from 5 % to 89 % of DK herds within the last 10 years.
- Microscopy of DD biopsies reveals spirochetes.
- Isolated spirochetes within genus *Treponema*:
  - ***T. phagedenis-like,***
  - ***T. pedis,***
  - ***T. brennaborense.***
  - ***T. vincentii-like***
  - ***T. denticola-like***

Digital dermatitis

- DD has increased from 5 % to 89 % of DK herds within the last 10 years.
- Associated with environmental conditions:
  - Loose-housing
  - Slurry / wet dirty feet
  - No grazing
- Rarely seen if tie-shed or all year pasture

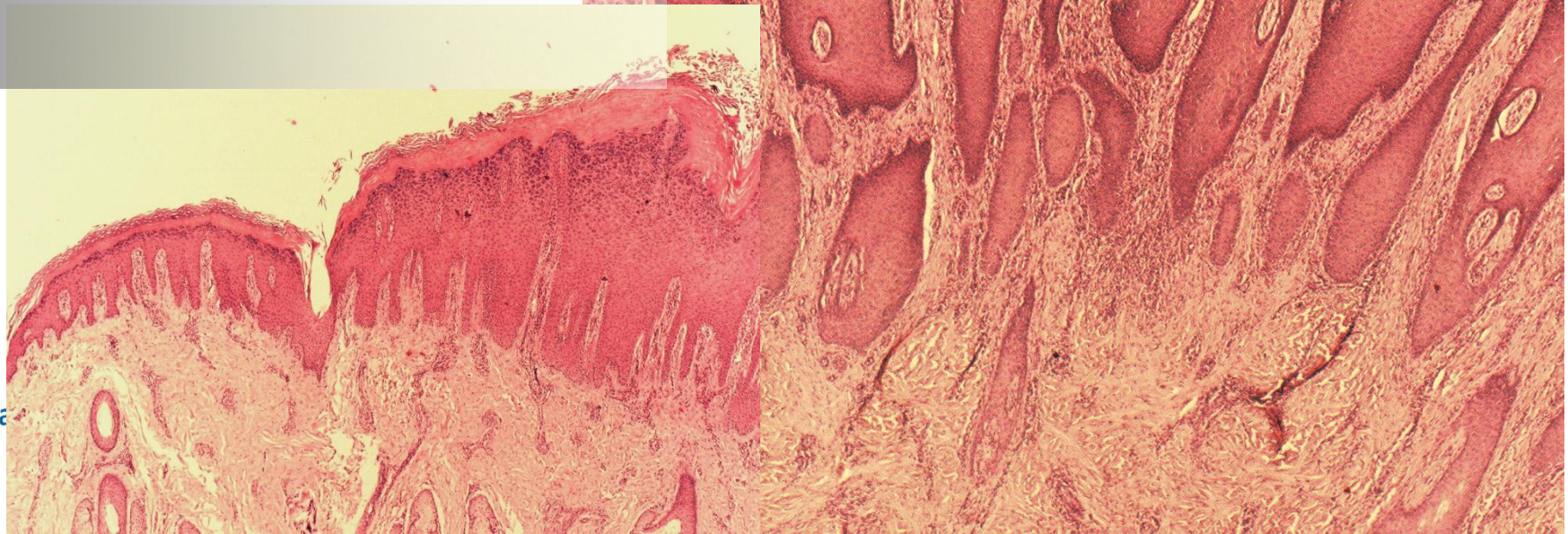


Danish farm house, 1780

Digital dermatitis

Histopathology

- Papillomatous proliferation of epidermis, acantosis, parakeratosis.
- Erosions and inflammatory infiltration.
- Perivascular dermatitis



Digital dermatitis

Aim: To investigate the etiology of DD by culture-independent molecular methods

Material: Skin biopsies from DD lesions collected from

1) a slaughter house

2) live animals

Slaughter house study (n= 41 biopsies) and

a) fresh tissue kept frozen until use

b) fixation in 10% buffered formalin, paraffin-embedded blocks for H&E and Fluorescenet in situ hybridization (FISH)

Digital dermatitis

Herd study including 5 herds and 73 biopsies

- 6 mm punch biopsies taken at routine hoof trimming
- The first 15 DD lesions sampled in each herd

Digital dermatitis

Methods:

- Purification of DNA from fresh lesions
- PCR amplification of the 16S rRNA gene
- Cloning of DNA
- Sequencing of randomly selected clones
- Phylogenetic analysis of sequencing result
- Design of oligonucleotide probes targeting 16S rRNA using the software ARB
- Fluorescent in situ hybridization in the formalin-fixed, paraffin-embedded tissue sections
  - PT specific probes(red) + probe for Domain bacterium(Eub)(green)
  - *Fusobacterium necrophorum, Dichelobacter nodosus, Guggenheimella bovis*

Digital dermatitis

Slaughter house study results I:

- Bacterial DNA obtained from 9 biopsies. Fragments of app. 1,5 kb were PCR amplified and cloned.
- A total of 237 randomly chosen clones were partially sequenced (600-700 bases). 122 belonged to genus Treponema. 50% of the remaining clones belonged to *Fusobacterium necrophorum*.
- 25 clones were full sequenced identifying 9 different phylotypes(PT).

Digital dermatitis

Slaughter house study results II:

- 41 lesions collected revealing DD in 40
- Polytreponemal infection in 39 biopsies by FISH
  - PT1 (95%), PT6 (93%), PT3 (85%) and PT2 (68%)
- *Fusobacterium necrophorum* detected in 22% of biopsies – only superficially.
- Other bacteria – rods and micro-colonies of cocci only seen superficially

Digital dermatitis

Herd study results I:

- Bacterial DNA obtained from 15 biopsies. Fragments of app. 1,5 kb were PCR amplified and cloned.
- A total of 235 randomly chosen clones were partially sequenced (600-700 bases). 101 belonged to genus *Treponema*.
- 8 clones were full sequenced identifying 5 new phylotypes.
- All together 17 different phylotypes were identified.

Slaughter house study

Herd study

Clone ID	Number of clones		Closest cultivable relative
PT1	**34**	**5**	**T. refringens (93%)**
PT2	**35**	**1**	**T. refringens (89%)**
PT3 DDKL-20*	**1**	**5**	**T. refringens (94%)**
PT4 DDKL-12*	**5**	**9**	**T. refringens (92%)**
PT5 DDKL-13*	**6**	**17**	***T. vincentii* (99%)**
PT6 DDKL-4*	**25**	**28**	***T. phagedenis* (98%)**
PT7	**12**	**10**	***T. denticola* (99%)**
PT8 DDKL-3*	**3**	**6**	**T. denticola (95%)**
PT9	**1**	**1**	**T. vincentii (96%)**
PT10		**1**	***T. brennaborense***
PT11		**8**	***T. pedis***
PT12		**1**	**T. refringens (94%)**
PT13		**3**	**T. refringens (90%)**
PT14		**2**	**T. denticola (92%)**
PT15		**1**	**T. refringens (93%)**
PT16		**1**	**T. denticola (94%)**
PT17		**2**	***T. refringens***

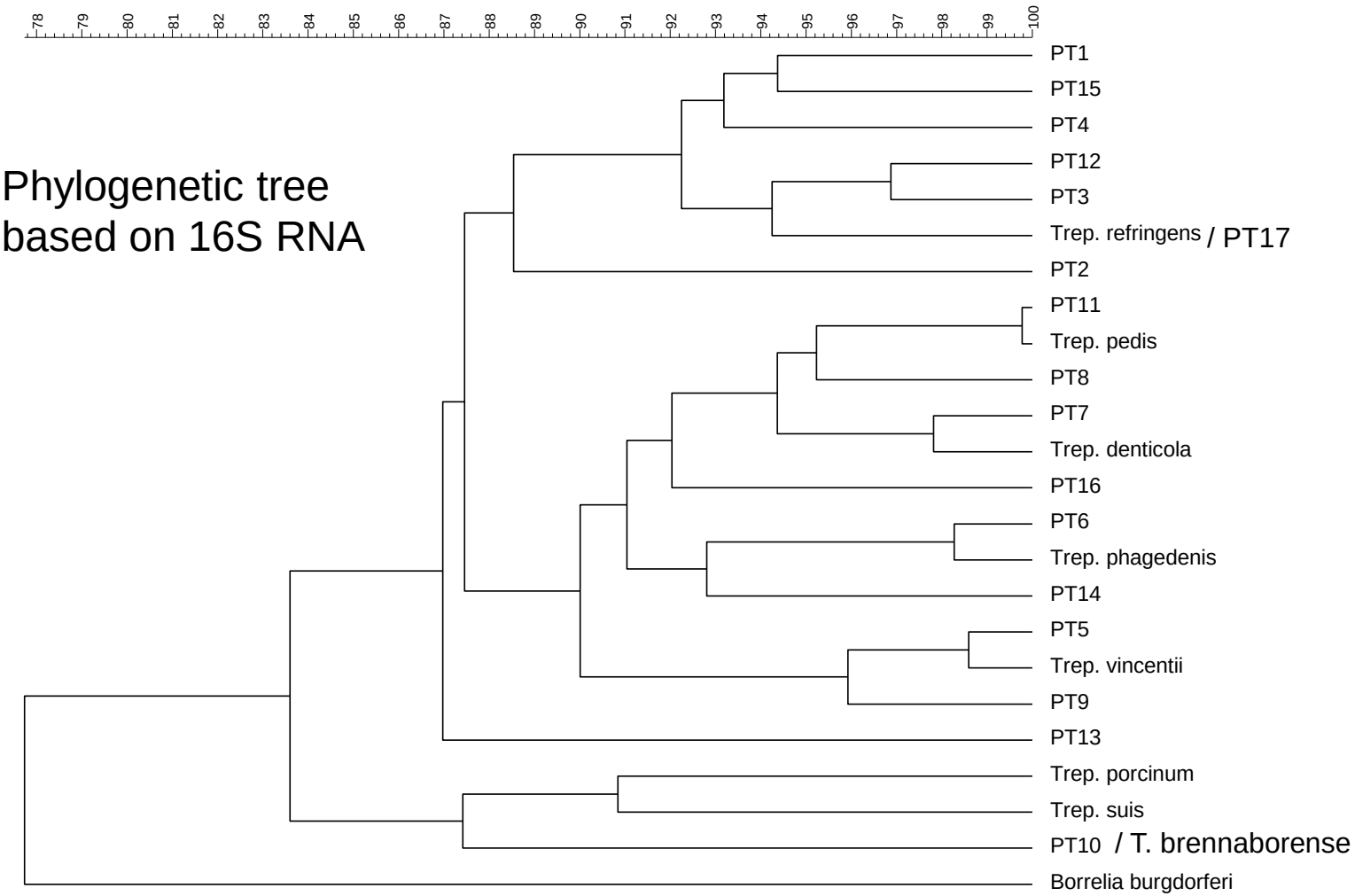
* Choi *et al. 1997*

National Veterinar

Pairwise (OG:100%,UG:0%) (FAST:2,10) Gapcost:0%
16srna

Digital dermatitis

Phylogenetic tree
based on 16S RNA



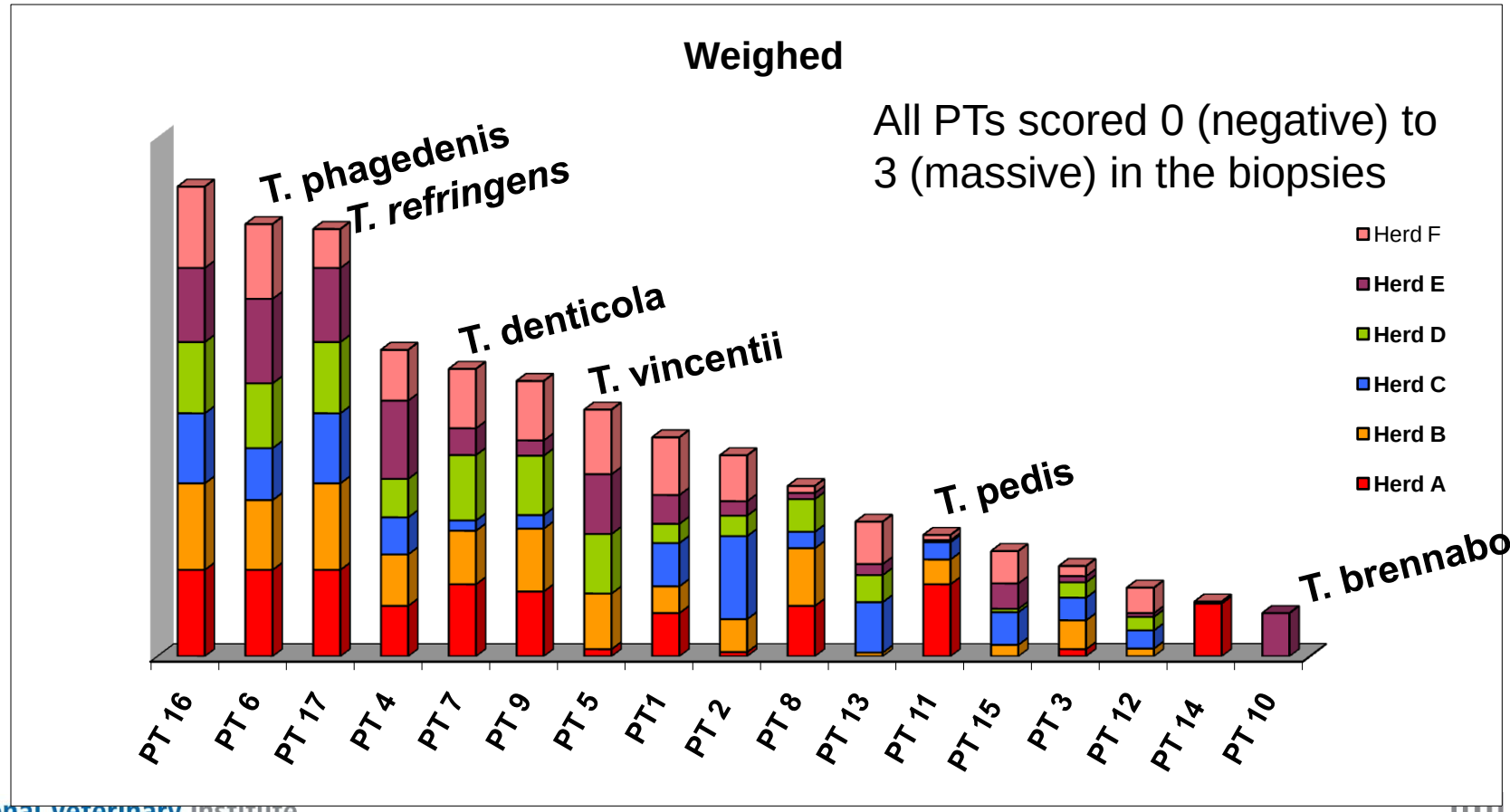
Digital dermatitis

Herd study results II:

- 85 lesions collected from 64 cows in 6 herds. All showing severe DD histopathologically.
- Polytreponemal infection found in all 6 herds: 14 to 16 PTs
- Intermingled polytreponemal infection found in all biopsies:
  Mean: A) 12.0, B) 12.0, C) 8.4, D) 11.7, E) 9.1 and F) 11.1
  - The most prevalent: PT16, PT6 (T. phagedenis), PT17 (T. refringens) and PT4
- In all biopsies the different PTs were scored 0 (negative) to 3 (massive).
- *D. nodosus* found in all herds in 20 to 73% of the biopsies.
- *Guggenheimella bovis* only rarely and superficially.

Digital dermatitis

Relative prevalence of Treponema phylotypes in DD biopsies from 6 herds



Digital dermatitis

Slaughter house and herd study results:

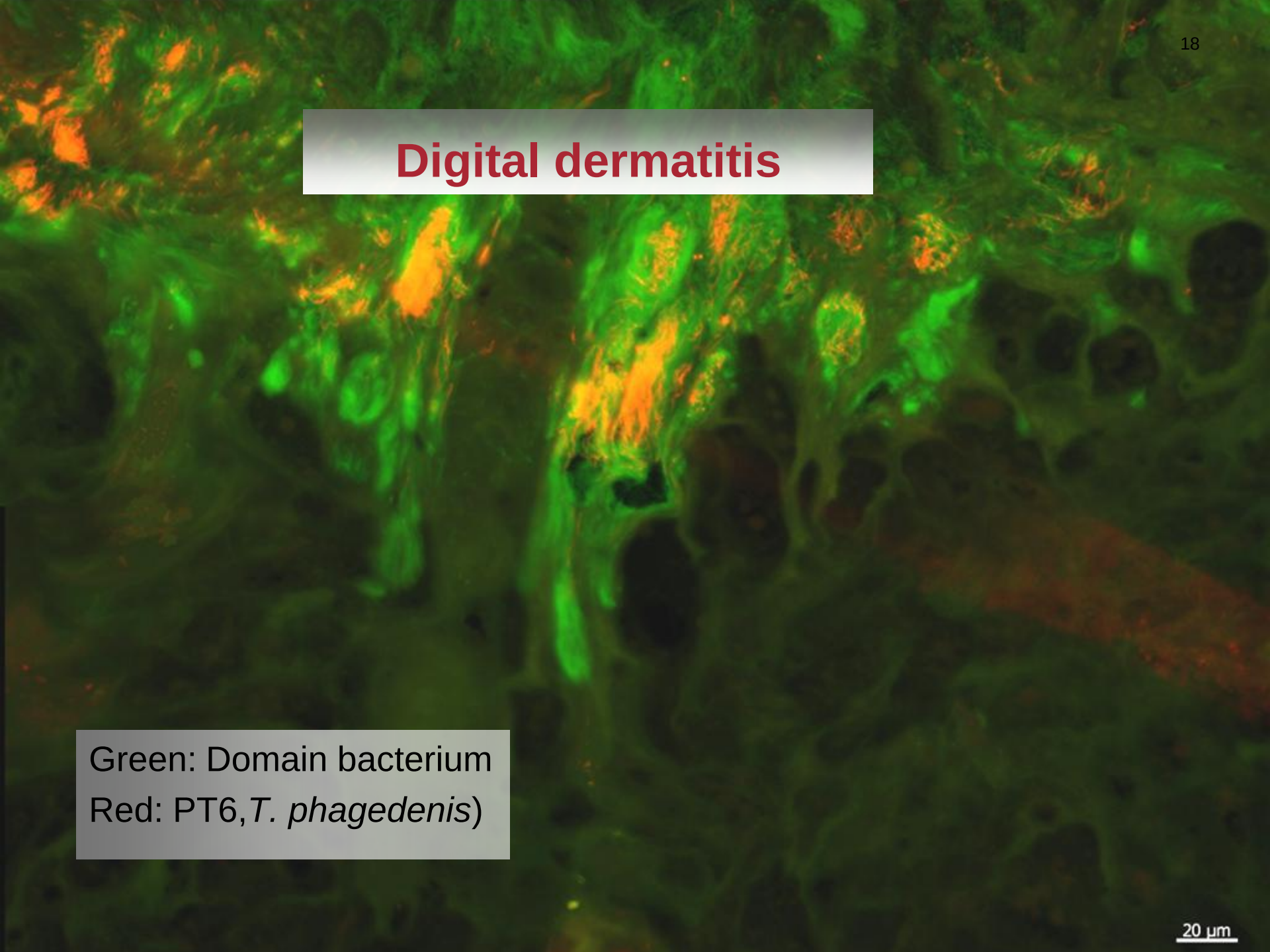
Slaughter house biopsies were mostly very chronic

Herd biopsies subacute to chronic

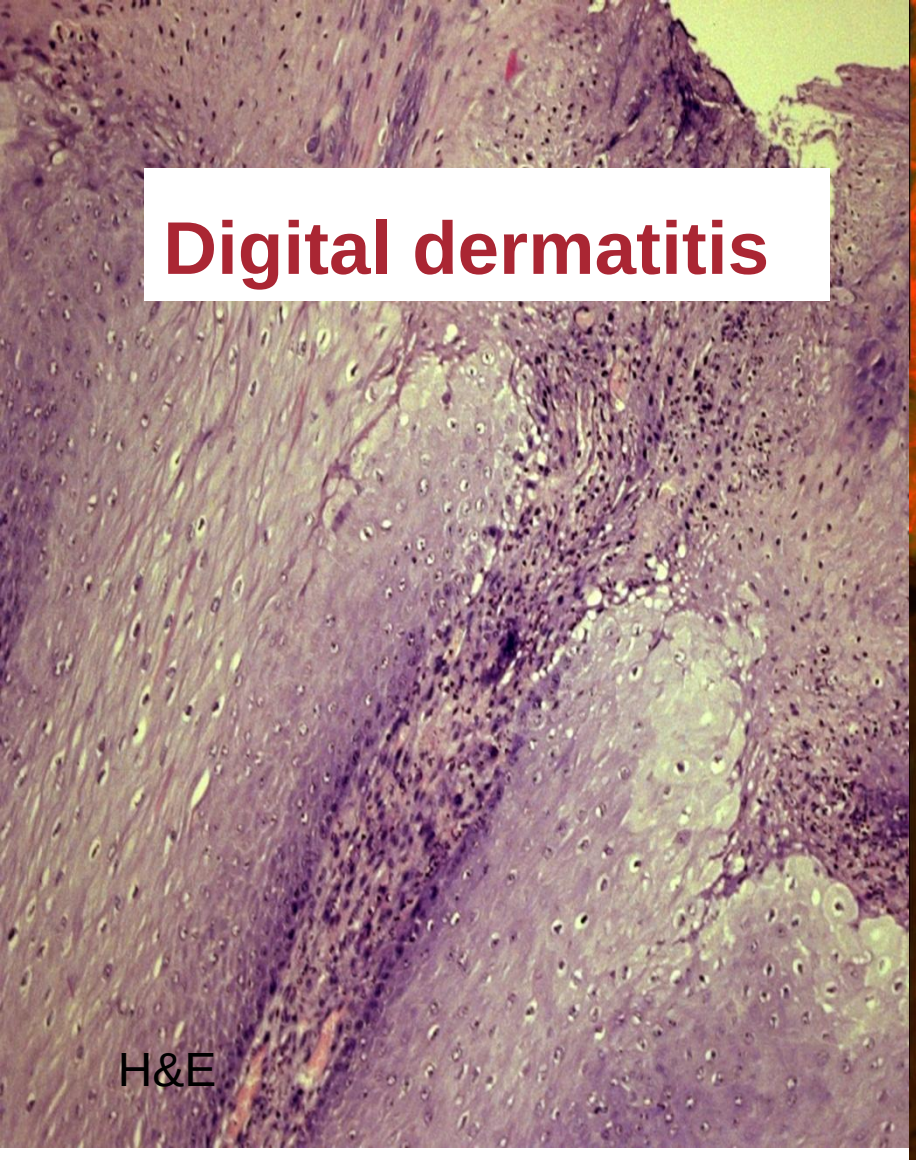
Bacteria other than spirochetes were seldom found deep in the lesion.

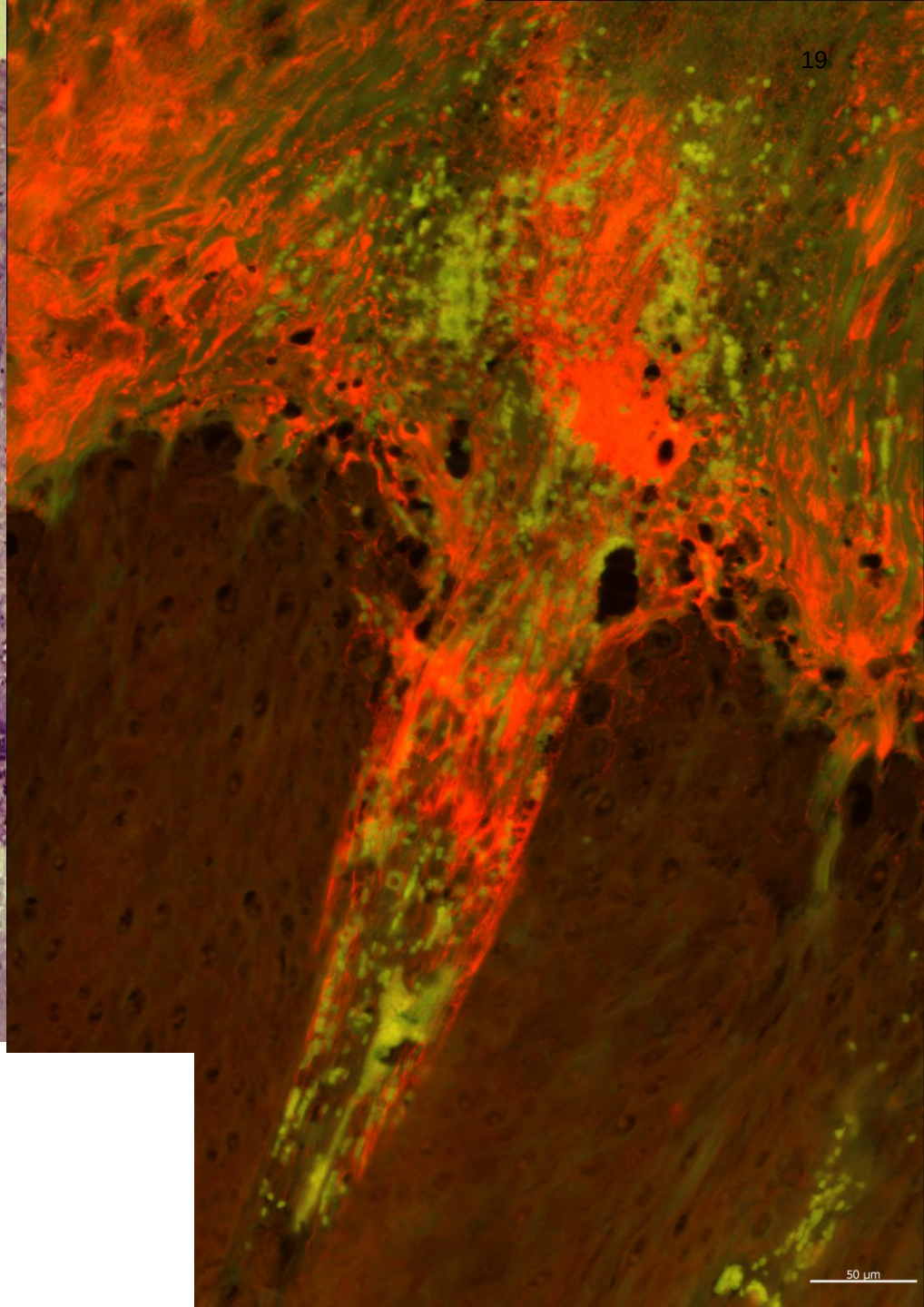
One of 8 control biopsies revealed subclinical DD lesions with D. nodosus and moderate treponemal infection. The 7 other biopsies were negative for *Treponema* as well as Domain bacterium

Digital dermatitis



Digital dermatitis

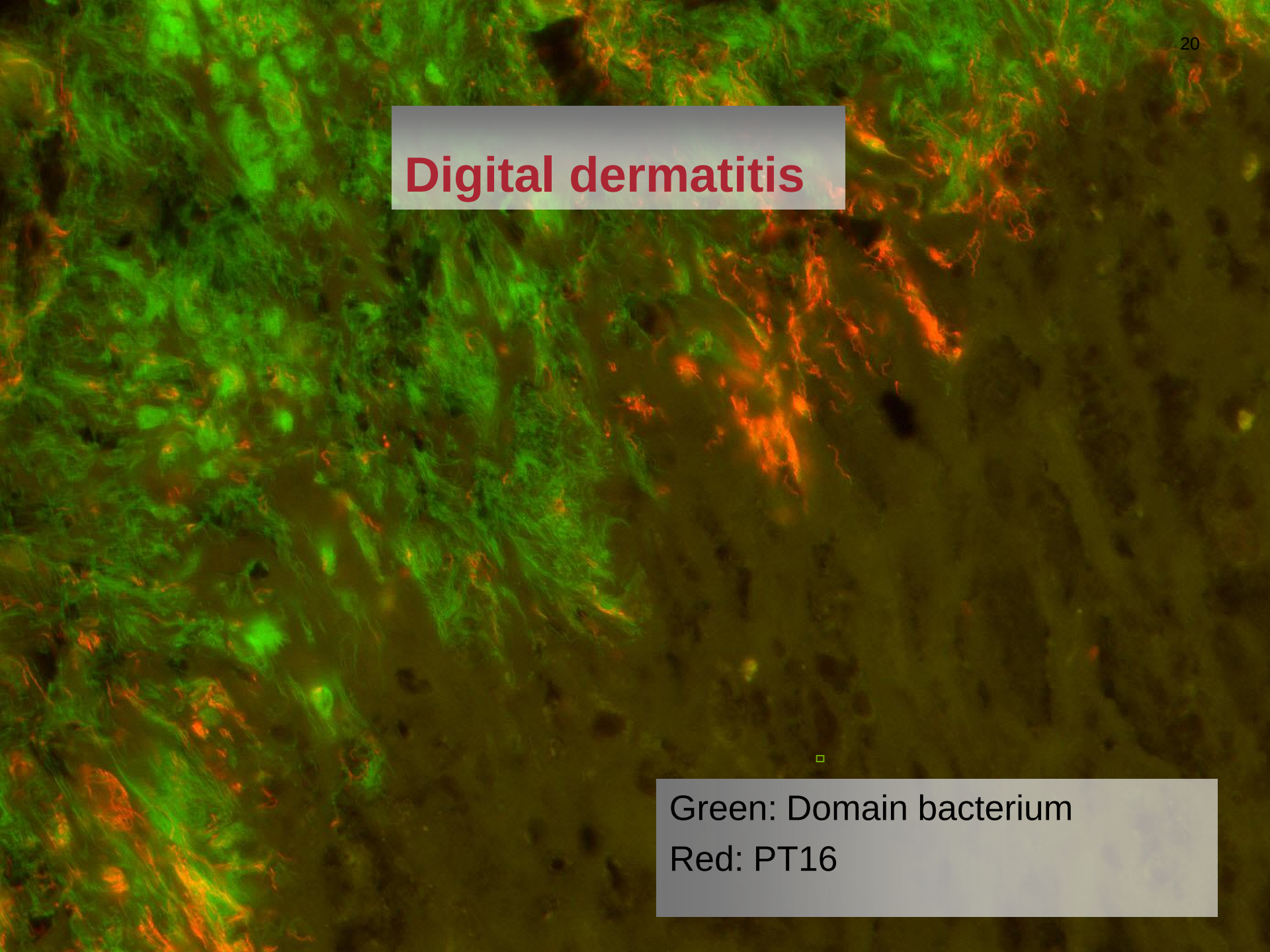




Green: Domain bacterium

Red: Treponema

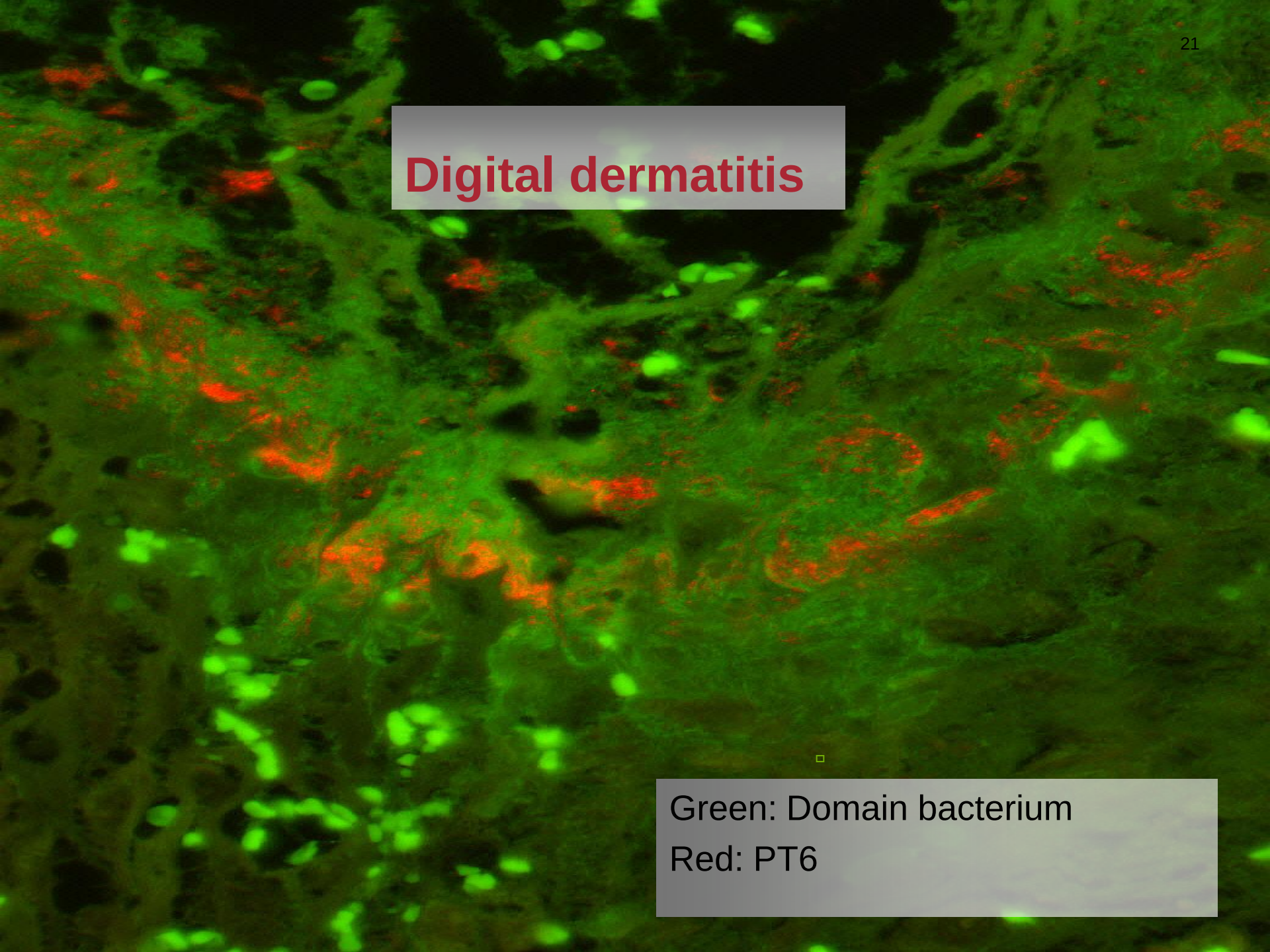
Digital dermatitis



Green: Domain bacterium

Red: PT16

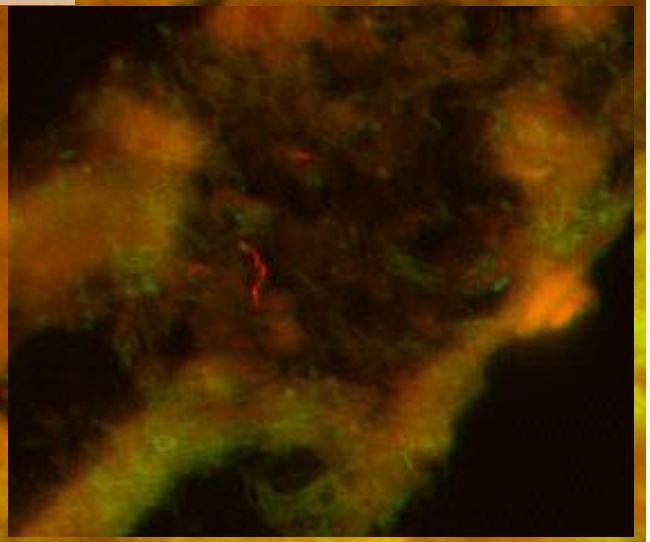
Digital dermatitis



Green: Domain bacterium

Red: PT6

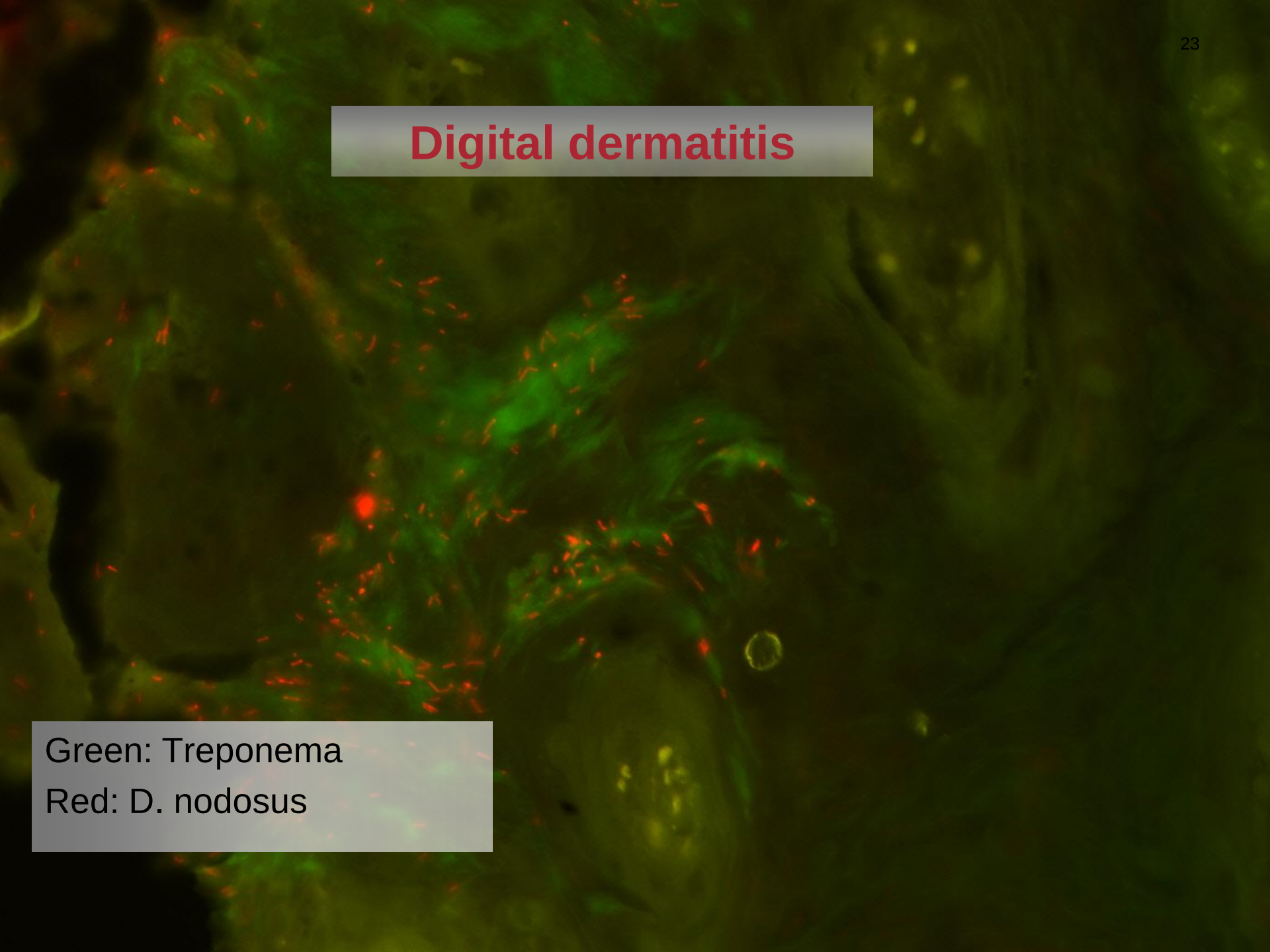
Digital dermatitis



Green: Domain bacterium

Red: PT13

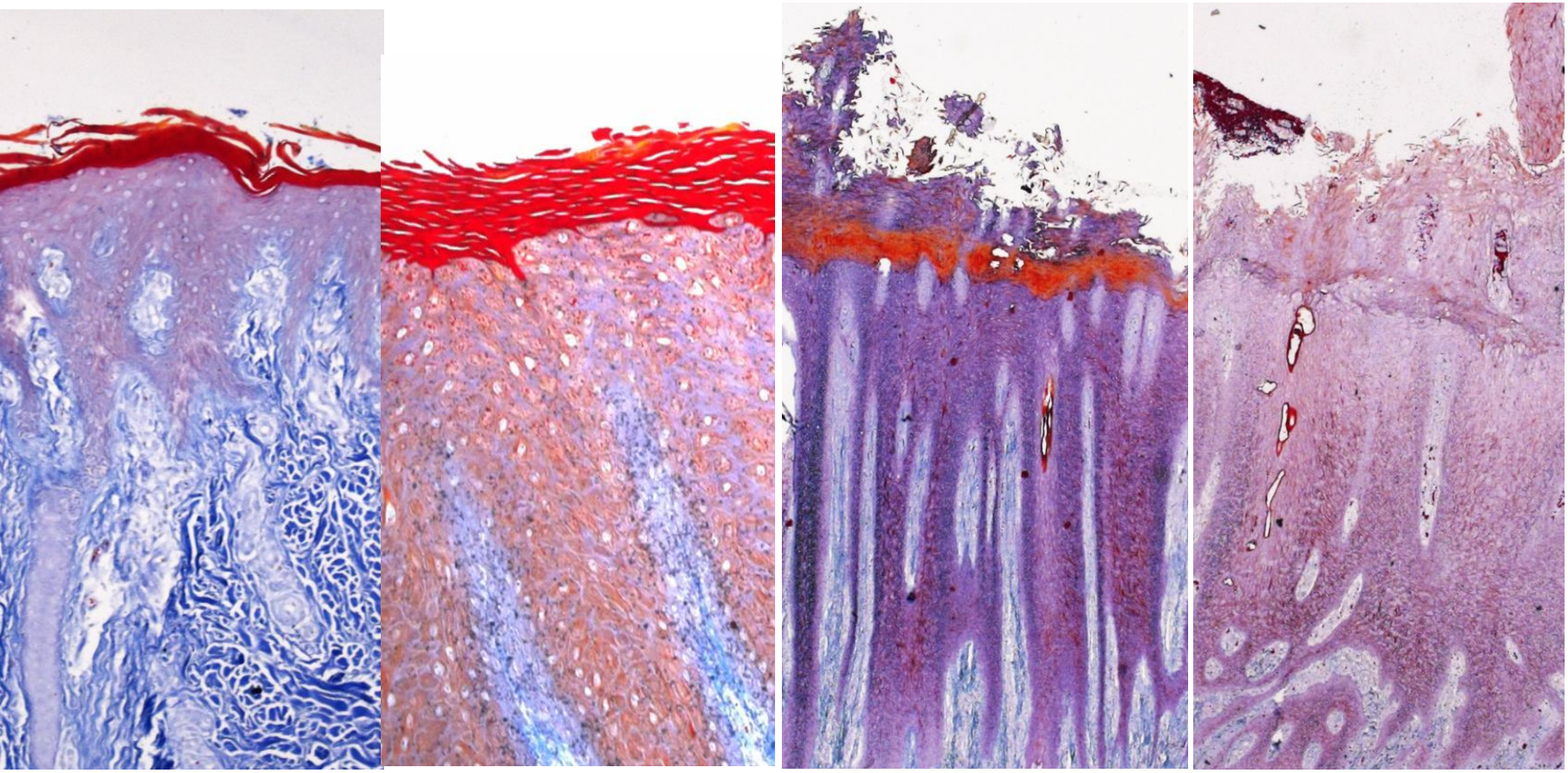
Digital dermatitis



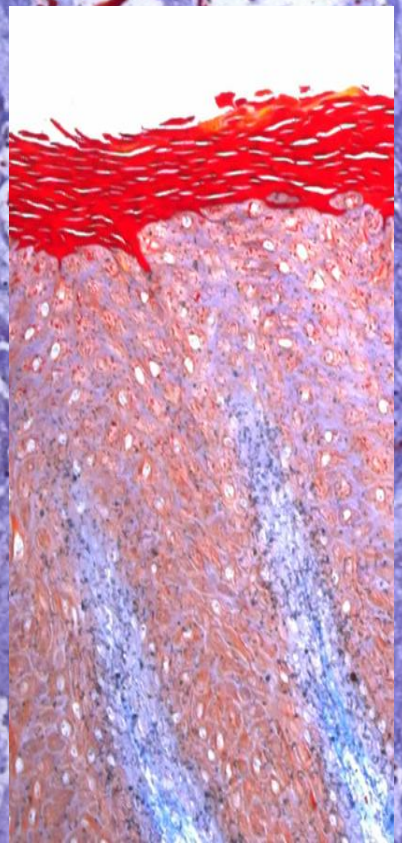
Green: Treponema

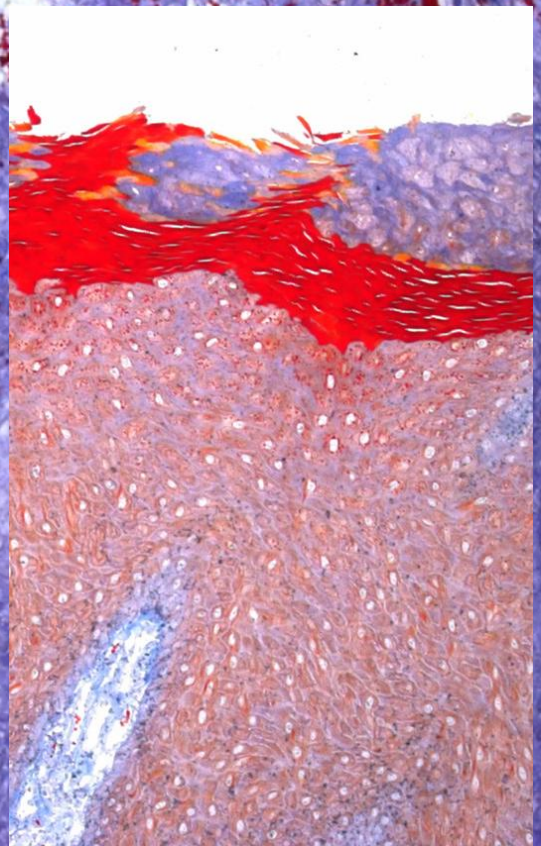
Red: D. nodosus

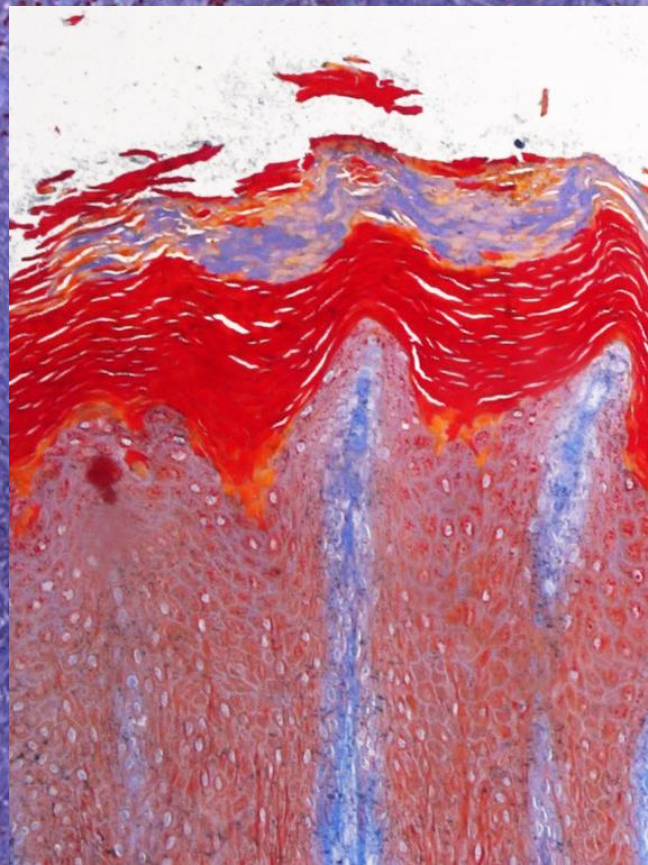
Digital dermatitis



Digital dermatitis







Digital dermatitis - overview

Table xx	Remarks	Epidermis		Fluorescent in situ hybridization				
Herd / animal ID		Score(0-3)/ keratin defects(x)	Thickness (µm)	Domain Bacterium	D. nodosus	F. necroph.	Trep score	Trep. phylotype
Danish herd A, n= 12	DD	Mean 3,0	ND	3,0	0,54	0,25	3,0	Mean 12,0
Danish herd B, n= 14	DD	Mean 2,9	ND	3,0	0,27	0,0	3,0	Mean 12,0
Danish herd C, n= 14	DD	Mean 2,5	ND	3,0	0,73	0,21	3,0	Mean 8,4
Danish herd D, n= 15	DD	Mean 3,0	ND	3,0	0,20	0,0	3,0	Mean 11,7
Danish herd E, n= 14	DD	Mean 3,0	ND	3,0	0,60	0,21	3,0	Mean 9,1
Danish herd F, n= 16	DD	Mean 2,9	ND	3,0	0,65	0,06	3,0	Mean 11,1
Norwegian herd G, cow 1	DD	2	ND	3	2	0	3	1, 3,
cow 2	DD	2	2358	3	2	0	3	1, 3
cow 3	DD	3	ND	3	2	0	3	1, 3
cow 4	DD	3	3845	3	2	0	3	3,
cow 5	DD	2	3941	3	2	0	3	3,
Non-affected cow 6	Subclinical DD	1	3031	1	1	0	1	3,
Non-affected cow 7	Hyperplastic	0	1202	0	0	0	0	
Non-affected cow 8	Normal skin	0	476	0	0	0	0	
Non-affected cow 9	Subclinical DD	1	2454	1	0	0	1	0
Non-affected cow 10	Hyperplastic	X	1419	0	0	0	0	
Norwegian herd H, 1-7	Hyperplastic	X	Mean 1543	0	0	0	0	
8-10	Hyperplastic	0	Mean 690	0	0	0	0	
Danish Sl. house* n=5	DD	3	Mean 3621	3	0,2	0,4	3	Mean 4,6
Healthy skin, Denmark Vet2	Subclinical DD	1	3648	3	2	1	3	1, 2, 12, 13, 14, 16
Vet1, KUL 1, 4, 7	Hyperplastic	X	Mean 1701	0	1/4	0	0	
KUL 2	Hyperplastic	0	781	0	0	0	0	
KUL 3, 6, 8	Normal skin	0	Mean 236	0	0	0	0	
Danish Sheep Affected skin	DD	3	>1800	3	2	3	2	3
Non-affected skin	Normal skin	0	170	0	0	0	0	

Digital dermatitis

Conclusion

- DD is closely associated with polytreponemal infection

  Mean: 8 to 12!!

  - So far acute, monotreponemal infection has not been detected!
- The most prevalent being: PT16 (100%), PT6 (*T. phagedenis*), PT17 (T. refringens) and PT4.

- Synergy between multiple *Treponema* species!!

- The treponema PTs are epitheliotropic – not dermatropic
- No other bacteria identified deep in DD lesions - suggesting that *Treponemas* someway are able to prevent proliferation of other bacteria
- Different PTs may colonize the same lesion successively in ”waves”

Digital dermatitis

Conclusion

- Infiltration by *Treponemas* presupposes damaged/lysed epidermis
- Demonstration of subclinical DD
  - A steady subclinical infection may aggravate into severe DD if new PTs are introduced or the environment changes.
- *D. nodosus* widespread in Dansh cattle and may be involved in DD.
- Fluorescent in situ hybridization usefull for identification in tissue samples

Digital dermatitis

Perspectives

- Genetic heterogeneity among isolates of *T. phagedenis*.

  Subtyping (PFGE, RAPD) shows considerable diversity among strains isolated not only from different cattle but also from the same individuals (Yano, 2009)

- DD treponemas closely related to human, oral treponemas – why? A common pathogenecity?

- Do the treponemas colonize other organs of the bovine?

  Intestines?

- Associated with ovine digital dermatitis!

Digital dermatitis

”Evidence of multiple *Treponema* phylotypes involved in bovine digital dermatitis as shown by 16S rDNA analysis and fluorescent in situ hybridization”

J. Clin. Microbiol. 2008, Vol.46. No 9. 3012-3020.

Thank you for your attention

Digital dermatitis

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Mette Boye

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